Biochemical examinations of the liver

Liver tissue

The liver plays an irreplaceable role in the intermediate and energy metabolism of carbohydrates , lipids and nitrogenous substances , ensures inactivation and excretion of endogenous substances and detoxification of exogenous substances, is the main site of synthesis of plasma proteins and coagulation factors .

Liver disease causes changes in numerous serum biochemical parameters . Laboratory tests providing information on various liver functions and are important for the diagnosis of liver disease. Based on their determination, the intensity of liver parenchymal damage can be estimated .

Knowledge of liver tissue morphology is essential to understand the interpretation of " liver test " results . About 72% are hepatocytes. Kupffer cells, endothelium and fat cells account for about 8% and 1% of the cells form the bile ducts . The rest of the total liver volume is extracellular fluid. The extent of damage to individual structures varies according to the type of disease. Acute liver damage (eg viral hepatitis) is characterized by disturbance of hepatocytes in particular. In other diseases, biliary outflow disorders associated with damage to bile capillary cells may predominate.

Biochemical examination of the liver

Biochemical tests for the examination of the liver and bile ducts can be divided into several groups:

Indicators of hepatocyte damage

See the Hepatocyte Damage Parameters page for more information.

As a result of damage to the cytoplasmic membrane, eg by inflammation or hypoxia, its permeability is increased and enzymes located in the cytoplasm are released into the extracellular space . The most sensitive indicator of hepatocyte membrane dysfunction is increased leakage of alanine aminotransferase (ALT) into the circulation. Other enzymes that enter the circulation from hepatocytes are the cytoplasmic isoenzyme aspartate aminotransferase (cAST) and the hepatic isoenzyme lactate dehydrogenase (LD 5). Increased activity of cytoplasmic enzymes in serum is a sign of reversible hepatocyte damage.

In more severe liver disease accompanied by hepatocyte breakdown (necrosis), not only cytoplasmic enzymes but also enzymes located in mitochondria enter the circulation . Glutamate dehydrogenase (GMD) and mitochondrial isoenzyme AST (mAST) are of particular diagnostic importance . The finding of increased activities of mitochondrial enzymes indicates irreversible, prognostically severe liver disease.

Indicators of cholestasis

Cholestasis, or congestion of the bile in the liver, can be caused by mechanical causes (biliary obstruction by a stone or tumor) or by a dysfunction, such as inflammation, some medications, or hereditary problems. Cholestasis is accompanied by an increase in the activity of enzymes found in the bile duct endothelial membrane and in the canalicular membrane of hepatocytes and a disorder in the outflow of bilirubin into the gut. Alkaline phosphatase (ALP) and γ-glutamyltransferase (GGT), blood and urine bilirubin , and urobilinogen are commonly investigated in clinical practice. in urine.

Indicators of liver biosynthetic functions

For more information, see Synthetic Liver Function Parameters page.

The intensity of proteosynthetic functions of the liver is mainly reflected by the state of the granular endoplasmic reticulum, where most plasma proteins are formed, but also by the synthesis of so-called "export proteins" such as some enzymes (eg cholinesterase) and coagulation factors . The determination of these analytes is particularly important in chronic liver disease. Unlike cytoplasmic and mitochondrial enzymes, whose activity increases with liver damage, a decrease in proteosynthesis results in a decrease in proteosynthesis. For diagnostic purposes, the determination of serum cholinesterase , serum albumin or transferrin and coagulation factors is mainly used .

Selected biochemical examinations in liver diseases

Aminotransferases:

Aminotransferases are enzymes that catalyze transamination, the essence of which is the transfer of an amino group from an amino acid to a keto acid and vice versa. From a clinical-biochemical point of view, alanine aminotransferase and aspartate aminotransferase are the most important .

Alanine aminotransferase:

Alanine aminotransferase (ALT , EC 2.6.1.2) catalyzes a transamination reaction in which the amino group is reversibly transferred from alanine to 2- oxoglutarate to form pyruvate and glutamate . The cofactor is pyridoxal-5 ´-phosphate.

ALT is found mostly in the liver , in other organs (skeletal muscle, myocardium and others) its activity is much lower. Unlike AST, it is localized only in the cytoplasm.

The ALT assay is a sensitive and relatively specific test for hepatocyte damage. Its activity in serum increases even with small damage to the liver cell, which is caused by increased permeability of the cytoplasmic membrane . In hepatitis (eg viral hepatitis), an increase in ALT is the earliest indicator of impaired hepatocyte membrane integrity. ALT monitoring is suitable for monitoring the course of the disease.

Physiological values of S-ALT:

Men up to 0.80 µkat / l Women up to 0.60 µkat / l

Aspartate aminotransferase:

Aspartate aminotransferase (AST , EC 2.6.1.1, English SGOT (serum glutamic-oxaloacetic transaminase) catalyses the reversible transfer of an amino group from aspartate to 2- oxoglutarate. Cofactors reaction is similar to ALT pyridoxal-5'-phosphate.

AST is more common in a number of organs - the liver, myocardium, skeletal muscle, kidney, pancreas and erythrocytes. It exists in the form of two isoenzymes - mitochondrial , which is present in mitochondria and represents about 70%, and cytoplasmic , located in the cytoplasm, which is represented by about 30%.

The cytoplasmic fraction is released into the circulation easily even with slight damage to hepatocytes, when the permeability of their cell membrane is disrupted. The mitochondrial fraction enters the blood only when the liver cell is necrosis (breaking down). A marked increase in serum AST activity is therefore a sign of hepatocyte breakdown, as both isoenzymes are released into the circulation.

Because AST is not specific only for liver tissue, its increase may be accompanied by damage to skeletal muscle and myocardium. AST rises in the blood in acute myocardial infarction and after heart surgery, but also after prolonged physical exertion. The determination of AST has a false positive effect on hemolysis , as it is present in relatively high amounts in erythrocytes.

Physiological values of S-AST:

Men up to 0.85 µkat / l

Women up to 0.60 µkat / l

Clinical-biochemical use:

Aminotransferases are significantly involved in the clinical-biochemical diagnosis of liver diseases.

For the acute viral hepatitis is characterized by multiple increase of the catalytic concentration of AST and ALT . The increase to 2-3 times the values is recorded already in the prodromal stage, culminating in the 7th-12th day after the onset of jaundice (maximum up to 100 µkat / l).

Normalization of levels is usually recorded 5. – 8. week. Significantly (on the order of ten times), but only for a short time, transaminases are increased in severe biliary colic. Other liver lesions are usually accompanied by a milder, up to five-fold increase in activity, and in chronic liver disease, transaminase activity is often just above the upper limit of the reference range.

In general, the rate of transaminase elevation well reflects the extent of liver damage. However, it should be borne in mind that with functional liver tissue loss, such as liver cirrhosis , the number of cells from which transaminases may be released may decrease to such an extent that serum transaminase activities fall within or just above the reference range. extensive liver lesions.

A slight non-specific increase in both transaminases can be encountered even after intense physical exertion (they are released from skeletal muscle) and also in obese people.

Methods for determination of ALT and AST:

To determine the concentration of catalytic activity of AST and ALT, a method based on the principle of Warburg optical test is recommended .

Aspartate aminotransferase:

The determination of AST activity uses two-step reactions.

Determination of AST catalytic concentration. MD - malate dehydrogenase:
https://www.wikiskripta.eu/w/Soubor:Stanoven%C3%AD_AST.png

In the first AST-catalyzed enzyme reaction present in the test sample, oxaloacetate is formed.

The resulting oxaloacetate is reduced to malate in a further indication reaction by the action of the enzyme malate dehydrogenase (MD) with the simultaneous oxidation of NADH to NAD + . AST activity is determined kinetically based on the rate of NADH loss during the reaction by measuring absorbance at 334, 340 or 365 nm. The catalytic concentration of AST is proportional to the decrease in absorbance.

In addition to the substrate (L- aspartate and 2- oxoglutarate), malate dehydrogenase and NADH, pyridoxal-5´-phosphate and lactate dehydrogenase are present in the reaction mixture for the determination of AST. The addition of pyridoxal-5´-phosphate ensures sufficient saturation of AST and thus ensures full activity of the enzyme. The presence of lactate dehydrogenase is necessary to ensure the reduction of endogenous pyruvate (present in the sample), which also consumes NADH, and thus to avoid obtaining falsely higher results. These reactions take place during a 5-15 minute pre-incubation of serum in the reaction mixture without 2-oxoglutarate.

After pre-incubation, we start the AST-catalyzed reaction by adding 2-oxoglutarate. After a short lag phase, ΔA is monitored by reading the absorbance at minute intervals for several minutes or by continuous monitoring. Since the initial absorbance of the reaction mixture is higher, it is recommended to take a reading against a blank test, which may be, for example, a solution of potassium dichromate.

Alanine aminotransferase:

The ALT assay, like the AST assay, is based on a two-step reaction.

The principle is the same as for AST , only alanine serves as an amino group donor instead of aspartate, and lactate dehydrogenase (LD) is used as an enzyme for the indication reaction, which also serves as an enzyme for the reduction of endogenous oxoacids.

Determination of ALT catalytic concentration. LD - lactate dehydrogenase: https://www.wikiskripta.eu/w/Soubor:Reakce_katalyzovan%C3%A1_GGT.png

Method:

In the first enzyme reaction, catalyzed by the alanine aminotransferase present in the sample, pyruvate is formed from alanine.

The resulting pyruvate is reduced to lactate in an indication reaction catalyzed by lactate dehydrogenase added together with NADH to the reaction mixture. The reduction of pyruvate to lactate is accompanied by a decrease in NADH, which results in a decrease in absorbance at 334, 340 or 365 nm. The catalytic concentration of ALT is proportional to the decrease in absorbance.

As with AST, the procedure requires a 5-15 minute preincubation of serum in the reaction mixture without 2-oxoglutarate. The reaction is started with 2-oxoglutarate.

γ-glutamyltransferase:

γ-glutamyltransferase (GGT , formerly also GMT, EC 2.3.2.2) is a key enzyme of the γ-glutamyl cycle , which ensures the transport of certain amino acids and peptides across the cell membrane from the extracellular fluid to cells.

GGT catalyzed reaction: https://www.wikiskripta.eu/w/Soubor:Reakce_katalyzovan%C3%A1_GGT.png

GGT catalyzes the transfer of the γ-glutamyl group : from γ-glutamyl peptides to other peptides, amino acids or water. The donor of the γ-glutamyl residue is the tripeptide glutathione (γ-glutamylcysteinylglycine) found in animal, plant and bacterial cells . Protects the body from oxidative stress (participates in the removal of hydrogen peroxide). It is restored by a reaction catalyzed by glutathione reductase.

Occurrence of GGT:

Cell membranes with high secretory or absorption capacity

Liver - microsomal fraction of hepatocytes and biliary lining cell membranes

Proximal renal tubules , enterocytes, pancreas

GGT synthesis is induced by some substances (barbiturates , antidepressants , alcohol). GGT can also be released from membranes by detergents, such as bile acids or alcohol.

γ-glutamyl cycle: https://www.wikiskripta.eu/w/Soubor:Gama-glutamylov%C3%BD_cyklus.png

Evaluation of serum GGT activity:

GGT elevations are typical primarily of hepatobiliary tract damage:

Intra-hepatic or extrahepatic cholestasis - in these cases alkaline phosphatase is also increased ;

Hepatocellular damage - acute and chronic liver diseases;

High isolated increase in GGT may be a sign of liver damage due to chronic alcohol consumption ;

Increased activity is in Alcoholics even if the liver is not yet damaged (induction of GGT synthesis);

Liver and pancreatic tumors.

Determination of GGT:

The principle of the determination is based on the reaction that GGT catalyses physiologically in the organism. The transfer of the γ-glutamyl residue from the substrate to the acceptor, which is glycylglycine, is monitored. L-γ-glutamyl-p-nitroanilide or L-γ-glutamyl-3-carboxy-4-nitroanilide is used as substrates. During the reaction, colored p-nitroaniline (from the substrate L-γ-glutamyl-p-nitroanilide) or 5-amino-2-nitrobenzoate (from the substrate L-γ-glutamyl-3-carboxy) is released after the transfer of the γ-glutamyl residue to the acceptor -4-nitroanilide), the increase of which is directly proportional to the GGT activity in the examined sample.

Physiological values of fS-GGT:

Men 0.14–0.84 µkat / l

Women 0.14–0.68 µkat / l

Physiologically, GGT levels are higher in men due to higher prostate levels .

Carbohydrate coefficient transferrin:

Until recently, γ-glutamyltransferase (GGT) was considered to be the best biochemical marker of one of the most common causes of liver damage, alcohol abuse. Currently, another parameter is added, carbohydrate deficient transferrin (CDT).

Transferrin usually contains four to six sialic acid residues in its molecule as a glycoprotein. Chronic alcohol abuse (60 g of alcohol per day for at least two to three weeks) increases the proportion of transferrin in the structure of which sialic acid is absent (0-2 sialic acids per molecule) - the so-called carbohydrate deficient transferrin (CDT).

It is considered a sign of chronic alcohol abuse if the CDT content exceeds 6% of the total transferrin. CDT levels remain elevated for about 2 weeks after the onset of abstinence.